Radiation-Induced Tissue Effects

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1. Radiation quality is important. The distribution and ionization density of HZE may result in different biological targets. That is, unique radiation ionization patterns lead to unique biological effects.

2. Tissues respond to radiation: Organized functional units are multicellular and respond to damage with a coordinated program directed towards the recovery of function of the tissue – NOT THE CELL.

3. Microenvironment (extracellular matrix and soluble proteins) is a target of radiation effects. These effects are:
   - Sensitive to low dose (0.1 Gy)
   - Rapid (within 1 hour)
   - Persistent (weeks)
   - Dynamic
   - Tissue specific
   - Radiation quality dependent

4. Central regulators operate to coordinate multicellular events: TGF-beta, etc.

5. Tissue integrity is as much a matter of signals as of survival.

(cf Castro et al., Radiation Research 2000)

The organization of multicellular organisms into purpose-specific tissue is obtained through differential expression of the genome; however, a cell receives information about how it should behave from its microenvironment, which consists of other cells, insoluble extracellular matrix (ECM) proteins, soluble hormones, and cytokines (1). In response to damage, the flow of information both locally between cells and tissues, and distantly between organs, is mediated in large part by cytokines (2). Tissue pathology and organ failure can arise from the lack of orchestrated communication between cells and among different cell types. Ionizing radiation damages individual cells; thus, one can argue that radiation response is the sum of individual cell responses, such as cell death. However, recent data support the view that tissues respond to radiation in a coordinated and multi factorial fashion and that radiation exposure ultimately compromises tissue integrity by altering the flow of information among cells (3). We have identified several general features:

1. Microenvironment is a target of radiation.
2. Tissue response to ionizing radiation is global yet innately tissue-and cell-type-specific. Thus, protein expression alterations in the mammary gland are distinct from those expressed in liver, and those in the mammary epithelium are distinct from those in the stroma.
3. Tissue response is evident very rapidly after radiation exposure.
4. Some proteins are clearly secondary to others, indicative of a dynamic network.
5. Tissue response can be detected after exposure to low whole body doses (0.1 Gy).

6. Radiation-induced changes can be persistent.
7. Microenvironment remodeling is radiation-quality dependent.

Tissue response to radiation is a composite of the results of genetic damage, cell loss and induced gene products. An integrated model of the varied and complicated cellular processes governing tissue response to radiation exposure could provide valuable indices of radiation susceptibility and clues for effective intervention.

Role of TGF-β

The cytokine TGF-β is a prime mediator of homeostasis in that it orchestrates multiple cell types in response to injury via its triple action controlling proliferation, apoptosis and ECM deposition and composition (4). The biological activity of TGF-β is constrained by its secretion as a latent complex, consisting of TGF-β non-covalently associated with its processed N-terminal pro-segment, called the latency-associated peptide (LAP). As with p53, post-translational modifications are critical regulatory events for TGF-β function *in vivo:* release from LAP is a prerequisite for TGF-β to bind to its cell surface receptors (5, 6). This event is called activation and acts as the switch to initiate tissue response to damage in several physiological processes, in particular inflammation, wounding, and angiogenesis (7, 8). We found that TGF-β immunoreactivity is significantly increased in the epithelium and stroma concomitant with decreased LAP immunoreactivity within 1 hr of irradiation (9). This reciprocal immunoreactivity shift is consistent with activation during which LAP is degraded, revealing previously masked regions of TGF-β. This pattern persists for up to 14 days after radiation, suggesting that there is a chronic stimulus for TGF-β activation. Functional evidence of radiation-induced TGF-β activation was obtained by administration of TGF-β neutralizing antibody to animals prior to irradiation, which specifically inhibits radiation-induced collagen III mRNA and protein, a known target of TGF-β (10). Radiation is the first exogenous stimulus known to cause latent TGF-β activation *in situ* (9).

Latent TGF-β activation demonstrates a quantitative linear dose response to γ- radiation exposures of 0.1 to 5 Gy in the mammary epithelium, while the adipose stroma exhibits a threshold of 0.5 Gy (10). For biological endpoints, high energy protons in the plateau region are usually considered to be similar to ^{60}Co gamma rays. Following whole body irradiation with non-Bragg peak 200 MeV protons at doses from 0.1-5 Gy, loss of latent TGF-β showed a threshold of 0.5 Gy in the adipose stroma with a concomitant induction of collagen III, while a graded dose response was evident from 0.1 Gy to 5 Gy without a threshold was evident in the epithelium (11). These findings are very similar to γ-irradiation, which indicates an RBE indistinguishable from 1 for this endpoint. However the onset, localization and chronicity of TGF-β activation were different in Fe-irradiated tissue compared to ^{60}Co-irradiated tissue (11). The earliest evidence of TGF-β activation after irradiation with 0.8 Gy 600 MeV Fe particles was at 3 hr, was predominantly epithelial, and persisted at least 14 days. The sensitivity of TGF-β activation to low dose radiation exposure and the rapidity with which activation is detected (9) suggests that latent TGF-β activation is one of the most responsive indices of tissue response to ionizing radiation. One mechanism of this sensitivity lies within the latent TGF-β. We have shown that efficient activation of latent TGF-β occurs in solution in a cell-free system by exposure to either ionizing radiation and metal-ion catalyzed ascorbate oxidation (12). These data indicate that latent TGF-β is both a sensor and signal for oxidative stress. The myriad roles that TGF-β plays during tissue response to damage suggest that TGF-β acts as an extracellular lynch pin, restrained as a latent complex but poised to direct multiple cellular responses and functions when events lead to activation.

Tissue Integrity

The response of cells and tissues to ionizing radiation depends on dose, dose rate, radiation quality, and context. Our recent studies have defined a rapid, programmed response to radiation that may be initiated at the cellular level but acts on the tissue. One of the consequences of this integrated multicellular response is remodeling of the extracellular matrix (ECM), which is mediated in part by the rapid activation of the cytokine, transforming growth factor β1 (TGF-β). We have shown that radiation quality affects the initiation, the composition and the duration of the microenvironment remodeling in mouse mammary gland, liver and skin. Future studies to understand the basic mechanisms of tissue response to radiation may provide leverage for manipulating radiation effects prospectively.

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